STUDIES ON ANTIBODIES AND IMMUNE GLOBULINS IN COLOSTRUM FROM DIFFERENT SPECIES AND THEIR TRANSFERENCE TO THE NEWBORN INFANT AND ANIMAL

BY

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ALMQVIST & WIKSELLS BOKTRYCKERI AB
UPPSALA 1958

Opbergs
iss. Uppsala
1958.

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AKADEMISK AVHANDLING

som med tillstånd av Medicinska Fakulteten vid
Uppsala Universitet för vinnande av medicine doktorsgrad
framlägges till offentlig granskning å Patologiska
Institutionens hörsal lördagen den 22 mars
1958 kl. 10 f. m.

av

FOLKE NORDBRING
Med. lic., Smål.

UPPSALA 1958
ALMQVIST & WIKSELLS BOKTRYCKERI AB

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Studies on Antibodies and Immune Globulins in Colostrum from Different Species and Their Transference to the Newborn Infant and Animal

by

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The present article comprises a summary of the following papers:

I. NORDBRING, F.: The appearance of antistreptolysin and antistaphylolysin in human colostrum. *Acta Pædiat. 46,* 481 (1957).

II. NORDBRING, F.: The failure of newborn premature infants to absorb antibodies from heterologous colostrum. *Acta Pædiat. 46,* 569 (1957).

III. NORDBRING, F.: The change in total nitrogen and electrophoretic pattern in human colostrum during the first days of lactation. *Acta Soc. med. upsal. 62,* 113 (1957).

IV. NORDBRING, F.: The change in total nitrogen, electrophoretic pattern and antibody titre in porcine and bovine colostrum during the first days of lactation. *Acta Soc. med. upsal. 62,* 135 (1957).

V. NORDBRING, F. and OLSSON, B.: Electrophoretic and immunological studies on sera of young pigs. I. Influence of ingestion of colostrum on protein pattern and antibody titre in sera from suckling pigs and the changes throughout lactation. *Acta Soc. med. upsal. 62,* 193 (1957).

VI. NORDBRING, F. and OLSSON, B.: Idem. II. The effect of feeding bovine trypsin inhibitor with porcine colostrum on the absorption of antibodies and immune globulins. *Acta Soc. med. upsal. 63,* 25 (1958).

VII. NORDBRING, F. and OLSSON, B.: Idem. III. Transfer of protein fractions and antibodies to the newborn pig by ingestion of porcine serum with a study of the effect of bovine trypsin inhibitor. *Acta Soc. med. upsal. 63,* 41 (1958).

In the following these papers will be referred to by the Roman numerals given above.

Introduction

The transference of antibodies from mother to offspring has been the subject of numerous studies, and distinct differences between species have been disclosed. The transmission may occur prenatally, or postnatally by way of colostrum, or a combination of these routes may prevail.

The human infant is born with a number of antibodies in his serum, provided that the mother has such antibodies in her serum. Although this rule is not applicable to a few antibodies, in general the prenatal route seems to dominate in man.

The relation in this respect between mother and offspring is entirely divergent in some animal species. Studies on newborn calves, lambs, kids, foals and, to a less extent, pigs have shown that these animals are devoid of antibodies at birth. Instead, the newborn animal acquires antibodies by ingestion of colostrum. Thus in the ruminants, the horse and the pig the colostral route prevails.

A great number of serum antibodies have been found to be associated with electrophoretic fractions, considered as γ-globulins (1, 7, 8, 10, 18). The antibody globulin may however constitute only a small part of the γ-globulin (7, 8). In agreement with the findings with regard to antibodies, the newborn human infant has a relatively high concentration of γ-globulin, while the animals lacking antibodies have extremely low concentrations of γ-globulin at birth, or no γ-globulin can be detected.

There are several observations, indicating that various antibodies appear in varying concentrations in human colostrum. Electrophoretic studies of human colostrum have revealed a predominance of globulins of low electrophoretic mobility, presumably carrying the antibody globulin. Similar studies on colostrum from the ruminants have revealed high concentrations of antibodies in colostrum from immunized animals, and a preponderance of slowly migrating globulins. In bovine colostrum, the antibody activity was found in these fractions, and therefore these globulins were designated immune lactoglobulins by Smith (17). The immune lactoglobulins resembled the serum globulin fractions of low mobility, although some differences were apparent.

In the following, the colostrum globulins of low electrophoretic mobility will be referred to as immune globulins, because this is convenient and generally accepted. It is however realised that the actual antibody content may account for only a very small portion of these fractions.

As was mentioned previously, it has long been known that the newborn domestic animal is able to absorb large amounts of antibodies from colostrum. With regard to the newborn human infant, an absorption of certain antibodies has so far been observed in only a few instances, mostly in experiments with feeding of antibody-containing serum, and the amounts of antibodies absorbed were apparently small.

The observation that the prenatal transmission of antibodies dominates in the human, and the evidence at present of a poor ability to absorb antibodies from the intestinal lumen by the human infant, would lead to the presumption that the antibodies of colostrum are of minor significance to the newborn infant. The function of human colostrum as a source of antibodies is however still much debated (2), and different opinions appear in various reviews and textbooks. Most authors believe that colostral antibodies are relatively unimportant to human infants (e.g. 16, 19), others flatly deny any importance of human colostrum in this respect (e.g. 5, 11), while some state that antibodies of colostrum probably are absorbed and are of significance (e.g. 6, 13, 15).

Since the opinions regarding the significance of colostral antibodies to newborn human infants are divergent, and since the investigations reported in the literature are relatively incomplete with scattered observations of an appearance of antibodies in mostly isolated colostrum specimens and a few absorption studies, the problem was taken up for study.

Plan of Investigation

It was considered important to determine the length of time after parturition during which antibodies are detectable in human colostrum. This is particularly important in view of the small volumes of colostrum, which are secreted and ingested during the first days. There is some evidence in the literature that the concentration of antibodies in human colostrum decreases as lactation proceeds, but studies in which the antibody concentration has been followed closely from day to day are very sparse. Therefore, the titres of certain antibodies were determined in series of daily samples of human colostrum from a number of women (I).

It was of interest to perform a similar investigation on colostrum samples from an animal species, in which a large volume of colostrum is produced from birth, and in which the colostral route of antibody transfer predominates. Therefore, the titre of certain antibodies was determined at intervals during the early lactation in colostrum from immunized sows (IV).

Because the antibodies seem to appear in the immune lactoglobulins, it was of interest to investigate the possible changes in concentration of these fractions in similar series of daily colostrum samples. This was performed by electrophoresis of human colostrum (III) and of porcine and bovine colostrum (IV).

In the majority of previous controlled experiments on the absorption of antibodies by the newborn human infant, serum containing antibodies has been fed. It was of interest to perform similar experi-

ments with feeding of colostrum. It would have been desirable to administer antibody-containing human colostrum, but no such colostrum was available so that heterologous colostrum had to be used (II). No attempt was carried out to measure electrophoretically the possible absorption of immune lactoglobulins by the human infant.

A comparison between the absorption capacity of the newborn human infant and that of an animal, highly dependent on supply of colostrum, would be of interest. The absorption of antibodies and immune globulins of colostrum by the newborn pig was studied (V, VI), as well as the absorption of serum antibodies and serum protein fractions (VII). Newborn piglets were chosen for the experiments, because these animals hitherto have been studied only incompletely, and because each pregnancy results in several newborn animals, available for study.

The discovery of a trypsin inhibitor in colostrum of different species by Laskowski and Laskowski in 1951 (9) suggested that this inhibitor might play a role in the mechanism of absorption of antibodies and immune globulins by protecting the protein from digestion. This is an attractive theory, and a study was carried out on pigs in order to determine whether such an effect could be demonstrated experimentally (VI, VII).

A. Antibodies in Colostrum

1. *Human Colostrum*

Since the main object of study was the day to day concentration of antibody in colostrum, it was desirable to obtain colostrum series from a large number of women and to select antibodies, which could be assumed to be commonly available. Antistreptolysin (AS) and antistaphylolysin (ASta) were chosen, because they are often present in sera from normal persons. These antibodies are seemingly unimportant in immunity against streptococcal or staphylococcal infections (4), but it was considered that they could be used as a test antibody for the general trend sought for. Control experiments revealed that these antibodies could be recovered in milk samples, and no unspecific inhibition of the lysin used in the tests could be observed (I).

The AS and ASta titres were determined in sera and in series of daily colostrum samples from 108 women (I). An AS titre of $\geqq 32$ units per ml was found in 98.1 per cent of the maternal sera, but only in 48.9 per cent of the colostrum samples of the first day post partum. An ASta titre of $\geqq 0.40$ units per ml was revealed in 92.6 per cent of the maternal sera, and in 38.3 per cent of the colostrum samples of the first day. In the great majority of cases the colostrum titres were considerably weaker than the titres of the corresponding maternal sera. In all series of colostrum samples, without exception and in-

dependent of the antibody level in the first day's colostrum, there was a rapid and marked fall in antibody titre from the first to the fifth day, occurring mainly during the first three days. No antibodies were found in the samples from the fourth and fifth day, except in a few specimens, having a low concentration.

It was thus shown that these antibodies appear in human colostrum only during a short time after birth.

2. *Porcine Colostrum*

Sows were immunized during gestation with a vaccine, prepared from *Salmonella paratyphi* A organisms, to obtain colostrum containing antibodies. Colostrum specimens were collected at intervals after birth from the sows, and the titre of H agglutinins was determined in these series of samples, using the Widal test (IV). Nine such series were available.

Agglutinins were found in high titres in the colostrum samples at parturition, sometimes in very high titres, and in all cases higher than in the sow's serum. The antibody titres diminished markedly in the course of a few days, the decrease occurring mainly during the first three days. The rate of disappearance of the agglutinins in porcine colostrum was thus found to be about the same as the rate of disappearance of the antilysins in human colostrum.

The general trait of an appearance of antibodies for only a short time after birth is thus common for the colostra from the two species. There is a difference between the species in volume of the colostrum secreted during the first few days. Women have a low production up to usually the third or fourth day, when often a sudden increase in volume occurs, while the yield of porcine colostrum is high already at birth, and the volume increases but slowly. The rapid disappearance of the antibodies thus occurs regardless of the different yields, and does not seem to depend on dilution but is merely a sign of a change in type of mammary secretion.

This raised the question, whether a similar rapid change in the protein composition of colostrum from different species could be revealed.

B. Nitrogen Concentration and Electrophoretic Pattern of Colostrum

1. *Human Colostrum*

Series of daily samples of colostrum, similar to those used for antibody analysis, were obtained from 20 women. The total nitrogen con-

centration was determined in the skimmed colostrum samples by micro-Kjeldahl analysis (III).

In all these series, there was a marked fall in nitrogen concentration during the first days of lactation, with the highest rate of decrease during the first three days. The mean total nitrogen concentration on the first post-partum day was 1620 mg per 100 ml, the mean concentration on the fourth day was 354, and on the fifth day 317.

The colostrum samples of eight series were prepared further, and the protein pattern of the wheys was studied by means of paper electrophoresis (III). The pattern of colostrum, secreted on the first one or two days, was characterized by a predominance of a large complex of globulins of low electrophoretic mobility. However, there was an obvious change in composition from day to day in all series studied, involving a decrease in the immune globulins. An approximate quantitative calculation of the concentrations of the globulins in question was carried out in two series, revealing a decline in both series from about 4 grams per 100 ml in colostrum of the first day to about 1 gram per 100 ml on the fourth day. The rate of decrease was almost identical with that obtained for the total nitrogen in the skimmed colostrum samples.

Thus, these studies have revealed not only a rapid and marked fall in nitrogen concentration of human colostrum from day to day of the early lactation, but also a change in protein composition. The decrease in the concentration of the immune globulins is coincident with the disappearance of the antilysins, and the short period of antibody appearance in human colostrum thus corresponds to an equally short period of predominance of the immune globulins. It is likely that the antilysins are localized in the immune globulin fraction. Although the antibody globulin might constitute only a small part of the immune globulins, the simultaneity of the decrease in globulins and disappearance of antibodies suggests that these two phenomena are connected.

2. *Porcine Colostrum*

Eleven series of porcine colostrum samples were obtained, corresponding to those used for the agglutinin analysis. The same investigations were carried out as in the case of human colostrum.

The total nitrogen concentration was very high in colostrum at birth, but subsequently there was a marked decrease in all series, the rate of decrease being highest during the first two or three days (IV). The mean nitrogen concentration at birth was 3190 mg per 100 ml, on the third day 766, and on the fourth day 747 mg per 100 ml.

Again, there was a preponderance of slowly migrating globulins in the colostrum, obtained soon after parturition (IV). A decrease in these components, similar to that in the human colostrum series, oc-

curred during the next few days in all the six series studied. The approximate concentration of the immune globulins was measured in one series of samples, and a drop in concentration was revealed from about 4 grams per 100 ml in the whey at farrowing to about 1 gram per 100 ml on the fourth day. The rate of decrease in immune globulins followed roughly that of the total nitrogen in the corresponding skimmed colostrum samples, and it occurred simultaneously with the decline in agglutinin titre.

A coincidence was thus again noted between the decrease in antibodies and in immune globulins, suggesting a relationship between these observations. By electrophoresis of porcine colostrum on a starch block, samples of the different protein fractions became available for agglutinin analysis (IV). It was demonstrated that the agglutinins were located in the immune globulins.

3. *Bovine Colostrum*

Four similar series of bovine colostrum samples were available, and the same analyses were carried out (IV).

There was a marked decrease in the concentration of total nitrogen during the first two or three days, and the predominant complex of immune globulins in colostrum at parturition decreased rapidly. Thus, the same trend was revealed, as that found in the studies of human and porcine colostrum. In one of the two series studied electrophoretically, the concentration of the immune globulins in the whey 12 hours post partum, about 7 grams per 100 ml, diminished to about 1 gram per 100 ml at 48 hours, and the concentration was only slightly lower on the fourth day.

No antibody analysis was performed in the series of cow's colostrum. However, there is evidence from the literature that a rapid decrease in antibody concentration occurs also in bovine colostrum.

Evidently, the rapid and coincident decline in antibody and immune globulin concentration is a characteristic trait in colostra from different species, and the rate of decrease is approximately the same in the different colostra.

Since the yield of colostrum is high already at birth in sows and cows, large quantities of antibodies and immune globulins are apparently available for the newborn piglet and calf. Since the period of secretion of colostrum with antibodies and a preponderance of immune globulins coincides with the period of poor yield in the human, small quantities of these constituents are apparently available for the newborn infant.

The next question is, whether the newborn infant is able to absorb the antibodies present in colostrum.

C. Absorption of Antibodies by the Newborn Human Infant

Most feeding experiments, reported in the literature, have failed to reveal an absorption, but occasionally about 0.1 per cent of the amount of serum antibodies administered have been absorbed. No doubt, very small amounts of protein are absorbed physiologically. Gruskay and Cooke (3), who fed egg albumin to human infants, estimated the absorption to 0.02 per cent of the dose given.

Bovine and porcine colostrum, containing paratyphoid H agglutinins, were given to nine premature infants (II). The feedings started soon after birth, because there is evidence from experiments on some animals that the passage of unaltered protein through the intestinal wall occurs mainly during a short period after birth. The agglutinin titre was determined in the colostrum fed and in sera of the infants, using the Widal test, and the amount of antibody absorbed was calculated from the data obtained. No quantitative estimation of the possible amount of antibodies absorbed by immunochemical methods was attempted, because it was soon realised that the rather crude method used was sufficient for the purpose of this investigation.

No agglutinins were found in any serum after feeding. It was calculated that the amount of antibody absorbed, if absorption had taken place at all, was less than 0.15–0.33 per cent of the amount given. Although the figures obtained are minimum figures, because the amounts of possibly absorbed antibodies, escaping into tissue spaces, have not been taken into account, it is apparent that the absorption ability of the newborn human infant is poor. This is particularly evident in view of the results of the feeding experiments on piglets.

D. Absorption of Antibodies and Immune Lactoglobulins by the Newborn Pig

1. *Suckling Pigs*

The content of total protein and non-protein nitrogen and the paper electrophoretic pattern of serum before (cord serum) and after normal suckling was studied on 56 pigs (V). The change in composition of serum was followed up to nine weeks of age. 11 of these pigs derived from immunized sows, and the H agglutinin titres of their sera was determined.

The porcine cord serum was found to have a unique composition.

The total protein concentration was only from 1.4 to 2.2 grams per 100 ml, while the non-protein nitrogen concentration was high (from 120 to 220 mg per 100 ml). The concentrations of albumin and γ-globulin were extremely low, the albumin being from 0.1 to 0.25, the γ-globulin from 0.1 to 0.2 gram per 100 ml. The β-globulin concentration was slightly higher (from 0.2 to 0.4 gram per 100 ml). The dominating electrophoretic component was the α_2-globulin (from 0.7 to 1.3 gram per 100 ml), probably composed of actual α_2-globulin and the mucoprotein, fetuin (14). Despite the low content of albumin, the piglets were not edematous at birth. A possible explanation is that the fetuin contributes to the plasma osmotic pressure. No agglutinins were found in the cord serum.

The ingestion of colostrum produced enormous changes in the sera of the pigs in the course of a few hours. The total protein increased roughly three-fold within 12 hours and remained at approximately the same level. Apparently, this increase was due to the passage of large amounts of slowly migrating globulins into the blood, because a large complex of γ- and β-globulins, difficult to separate, appeared in the sera. This complex had its peak on the first day of life, amounting to about 70 per cent of the total protein. Coincident with the appearance of the $\beta+\gamma$-globulin complex, agglutinins were found in the sera of the pigs, ingesting antibody-containing colostrum. No significant increase in titre occurred after 12 hours of age. Evidently, the absorption of the antibody-containing immune globulins occurs very rapidly.

A steady decline in γ-globulin was noted after the first day of life. This decline continued to the age of about four weeks. Subsequently, the γ-globulin level was stationary or increased slightly, while the antibody titre continued to decrease. Obviously, the animal's rate of synthesis of its own γ-globulin equals the rate of catabolism of the passively acquired γ-globulin at the age of about four weeks.

The change in the albumin concentration followed an entirely different pattern with a continuous, slow increase. This seems to be a manifestation of a synthesis by the animal itself, and the increase in albumin compensates for the decrease in γ-globulin.

The changes in the α-globulin fractions were found to be fairly small. An interesting observation was the prompt fall in the non-protein nitrogen concentration, as soon as the complex of $\beta + \gamma$-globulins appeared in serum, suggesting that the nitrogenous fractions, designated as non-protein, were combined with the immune globulins absorbed. If the absorption was low or none, the non-protein nitrogen level remained high (VI).

The results of these studies thus have shown that the newborn pig is able to absorb large amounts of colostral antibodies and immune globulins easily and rapidly. An attempt was made to measure the amounts absorbed by feeding known quantities.

2. *Pigs Fed Porcine Colostrum in Known Quantities*

Forty-four piglets were given porcine colostrum, containing paratyphoid H agglutinins, by stomach tube (VI). The feedings started at different intervals after birth. The same analyses as previously were carried out. The increase in $\beta + \gamma$-globulins after feeding was expressed as a percentage of the amount of immune globulins given. The amount of agglutinins absorbed was calculated in the same manner as in the experiments on human infants (II). All these figures represent minimum values, because only antibodies and immune globulins, appearing in the circulating blood, have been taken into account.

The relative increase in the slowly migrating globulins, when feeding of colostrum was started immediately after birth, corresponded to 17 per cent of the amount of immune globulins administered. An absorption did occur, even when the feeding was postponed for 72 hours, but the absorption figure was considerably lower, being 4 per cent.

The agglutinin absorption figures varied from 4 to 21 per cent of the amounts of agglutinins administered, when colostrum was given from birth. Corresponding values, when colostrum was given from 72 hours, were from 0.2 to 3 per cent.

Although the figures of the agglutinin absorption are not entirely comparable with those obtained in the studies on human infants, the difference between the species in absorption ability is nevertheless quite obvious, the ability being far greater in the pig than in the human infant. It is of interest that newborn piglets absorb antibodies from heterologous colostrum as easily as from homologous colostrum (12).

Because serum was fed in the majority of previous experiments on human infants, it was of interest to investigate the absorption of serum protein fractions and antibodies by the newborn piglet.

3. *Pigs Fed Porcine Serum in Known Quantities*

Thirty-one piglets were given agglutinin-containing porcine serum in the same manner as in the colostrum experiments and with the same analyses (VII). The increase in γ- and β-globulin and in albumin, relative to the amount administered, was calculated as previously, as well as the absorption of antibodies.

Antibodies were found in sera from all animals, given serum from birth, and the absorption figures varied from 4 to 15 per cent of the amount administered. Antibodies were also absorbed, when the feeding was started at 26 hours of age, but not at 40 hours.

The highest relative increase occurred in the γ-globulin, corresponding to 10 per cent of the amount fed at birth. The values for β-globulin and albumin were 5 and 4 per cent, respectively. Small changes occurred, when serum was fed from the age of 40 hours.

The absorption of slowly migrating globulins of serum was compared with the absorption of immune globulins of colostrum. A figure was obtained, which was half that of the colostrum globulin absorption. However, approximately twice the amount of globulins were given in the colostrum experiments as in the serum experiments, and therefore the absorption from serum might be as good as the absorption from colostrum.

4. *The Effect of Addition of Bovine Trypsin Inhibitor to the Feed on the Absorption of Immune Globulins*

The possible influence of a trypsin inhibitor, added to the feed, on the absorption was also investigated in the colostrum and serum feeding experiments on pigs (VI, VII).

Trypsin inhibitor was prepared from large amounts of bovine colostrum. The litters of pigs in each experiment were divided into two groups, one receiving colostrum or serum with an excess of inhibitor of high activity added, the other receiving only colostrum or serum. The amounts of the protein fractions absorbed in each group were compared, and the differences between the groups were analysed statistically.

It was found that the mean values of the relative increase in protein were higher in the groups, receiving inhibitor, in both the colostrum and serum experiments, and the difference between the groups was probably significant. The presence of a trypsin inhibitor in the feed thus seems to enhance the possibility of protein molecules to pass unaltered through the gastrointestinal wall. However, regardless of the inhibitor, there is a change in intestinal permeability with increasing age (VI). The explanation of this change is not known.

Conclusions

The situation for the newborn piglet is: there is a high yield of colostrum, and the animal is able to utilize the large supply of immune globulins and antibodies, because it has a good capacity of absorption. The presence of a trypsin inhibitor in colostrum seems to increase the amount of protein absorbed. The short duration of the period of secretion of colostrum is quite sufficient, because large amounts of immune globulins and antibodies are absorbed in the course of a few hours.

The situation for the newborn human infant is: the period of secretion of colostrum with antibodies and a high concentration of immune globulins is equally short and coincides with the period of low yield. The supply of antibodies and immune globulins is thus much less than

that available to the piglet. Moreover, the capacity of absorption is poor, and only minimal amounts may be absorbed.

The results of this comparative study illustrate the differences between the species, the colostrum being of vital importance to the newborn piglet but apparently of little significance to the newborn human infant.

Acknowledgements

My sincere thanks are due to the staff and personnel of the Department of Pediatrics, the Department of Gynecology and Obstetrics, the Institute of Hygiene and Bacteriology, and the Department of Clinical Chemistry of Uppsala University; and the Department of Animal Nutrition, Genetics and Hygiene of the Royal Veterinary College, Stockholm, for kind support and cooperation. For valuable advice and criticism in numerous discussions, I am greatly indebted particularly to Professor Bo Vahlquist, M.D., who introduced me to this subject, to Professor Gunnar Löfström, M.D. and Professor Anders Grönwall, M.D., who put their laboratories at my disposal, and to Professor Olof Mellander, M.D., Laborator Gunnar Laurell, M.D., Docent Stig Sjölin, M.D. and Docent Gunnar Wallenius, M.D.

I am grateful to Professor John Næslund for allowing me to obtain material for investigation.

I also wish to thank Mrs. Maj-Lis Johansson, Mrs. Marga Jahnke and Dr. Joachim Riebe for excellent technical assistance; Mr. Avo Raud, Fil. Kand., for advice concerning the statistical considerations of the results; Dr. John Glomset for revision of the English text; and Mrs. Karin Norrman for drawing the diagrams.

The work was supported by grants from the Faculty of Medicine and the Regnell Fund, Uppsala University.

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